

U.S. Serial No. 09/830,080
Page 3 of 20

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-26. (Cancelled)

27. (New) A method of affinity separation, comprising the steps of:

(a) providing a sample containing a target analyte;
(b) providing a matrix comprising an immobilized protein, said immobilized protein comprising one or more modifications that (i) increase the stability of said protein in alkaline conditions, and (ii) permit said protein to bind to said target analyte, said one or more modifications selected from the group consisting of:

- (1) deleting one or more Asn residues in said protein;
- (2) substituting one or more Asn residues in said protein for an amino acid that is less sensitive to alkaline conditions;

U.S. Serial No. 09/830,080
Page 4 of 20

(3) chemically modifying one or more Asn residues in said protein; and

(4) combinations thereof;

(c) contacting said sample and said matrix, wherein said target analyte binds to said immobilized protein; and

(d) isolating said target analyte from said matrix.

28. (New) The method of claim 27, wherein two or more Asn residues are modified.

29. (New) The method of claim 27, wherein all Asn residues are modified.

30. (New) The method of claim 27, wherein said Asn residues are replaced with an amino acid selected from the group consisting of lysine, aspartic acid, leucine, and combinations thereof.

31. (New) The method of claim 27, wherein said modifications are on the surface of said protein.

32. (New) The method of claim 27, wherein said immobilized protein comprises Albumin-Binding Protein (ABD).

U.S. Serial No. 09/830,080
Page 5 of 20

33. The method of claim 32, wherein said modifications in said Albumin-Binding Protein (ABD) are selected from the group consisting of (i) Asn₂₈ replaced by Leu, (ii) Asn₄₂ replaced by Asp, (iii) Asn₄₅ replaced by Asp, (iv) Asn₄₆ replaced by Lys, and combinations thereof.
34. (New) The method of claim 27, wherein said immobilized protein is a combinatorial protein.
35. (New) The method of claim 34, wherein said combinatorial protein is derived from an immunoglobulin molecule, staphylococcal protein A (SPA), or a DNA binding protein.
36. (New) The method of claim 34, wherein said combinatorial protein comprises domain Z of the B domain of SPA.